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(54) Title: HAEMOSTATIC BACTERICIDAL COMPOSITIONS

(57) Abstract: The invention relates to a local preparation having combined haemostatic and bactericidal effect. The haemostatic preparations comprise, as active haemostatic and bactericidal ingredients, a polyelectrolyte matrix of a polymeric carboxylic acid of a predetermined average molecular weight range and a bactericidal agent, wherein bactericidal agent is silver in the form of dissolved, free silver ions, oligomeric silver clusters, silver colloidal nanoparticles or any combination of such. Methods of production of these haemostatic preparations on the basis of poly(acrylic acid) of a predetermined average molecular weight range by introducing silver salts into the aforementioned polymeric acid, adjusting pH of the solution and irradiation by light, where applicable, are described. The compositions may be liquid or dry.

Haemostatic Bactericidal Compositions

Field of the Invention

The present invention relates to medical and veterinary preparations, specifically to haemostatic preparations for stopping haemorrhaging in various tissues and organs, which may additionally have bactericidal and sustained release bactericidal effects.

Background of the Invention

There exists a range of known haemostatic preparations of both natural and artificial origin. Haemostatic preparations of vegetable origin include. for example, *Lagochilus inebrians Bunge* (inebriant harelip), which is useful for localized treatment [see M.D. Mashkovsky; *Lekarstvennyie Sredstva* (Medicinal preparations), Moscow, "Medicine", 1985, pages 99, 100]. Synthetic haemostatics for local use, are, for example, grafted co-polymers of cellulose with α,β-unsaturated carboxylic acids, such as acrylic and methacrylic acids, processed with a solution of metal hydroxide or weak acid for achieving greater mechanical strength (USSR Authorship Certificate No. 219594).

Natural haemostatic preparations are susceptible to poor supply and are costly due to limited habitat. The synthetic preparations are insoluble in water and can thus only be used in the form of dressings. Available local haemostatics do not have bactericidal properties, and severe infection is a common problem. The application of incomplete iron salt of poly(acrylic acid) as a local haemostatic (Feracryl) is known (see Russian Patent No. 698,622, FR2,426,469 US Patent No. 4,215,106). This preparation provides haemostasis of both normal and pathological blood, such as in haemophilia, Wehrholf's disease, etc. To stop haemorrhage, gauze pads soaked in 1% Feracryl solution, dried and passed through an autoclave are used. Applying such a pad to a wound surface stops bleeding. A major drawback

of this preparation lies in its insufficient bactericidal activity, which may lead to wound infection, especially in warm and damp climates.

Other haemostatic preparations have been described. Of particular note is Russian Patent No. 2,056,843, disclosing a preparation having anaesthetical properties. This contains a complex of poly(acrylic acid) and a suitable anesthetic (such as novocain or lidocain). However, this complex preparation has poor bactericidal activity and short shelf-life.

US Patent No. 6,004,546 discloses the use of an essentially water-insoluble compound of bismuth and poly(acrylic acid) as a good mucoadhesive covering of the bowel wall, for treatment of patients with inflammatory bowel disease. The purpose of the preparation is to maintain bismuth in intimate contact with the inflamed tissue of the bowel, no haemostatic or antiseptic effects being disclosed.

A metal-containing, polyacrylate-based haemostatic has been described in unpublished Israel Patent Application No. 129,902, now abandoned).

WO99/04828 discloses use of hyaluronic acid derivatives in the preparation of biomaterials with a physical haemostatic and plugging activity and a preventive activity in the formation of adhesions following anastomosis.

It is an object of the present invention to provide a haemostatic preparation that also affords antibacterial protection and may be easily and economically synthesised.

Another object of the present invention is to provide such antibacterial haemostatic preparation that has a long shelf-life.

Another object of the present invention to provide a new effective local haemostatic which would coagulate blood proteins and thus produce a haemostatic effect in both normal and damaged blood coagulation systems.

A further object of the present invention is an antibacterial haemostatic preparation which provides for prolonged release of the bactericidal agent.

Yet another object of the present invention is a simpler and more controllable synthetic procedure of the preparation and simplified sterilisation.

Yet another object of the present invention is the conservation of the product by drying, particularly freeze-drying.

Other objects of the present invention will be clear from the detailed description thereof.

Summary of the Invention

In a first embodiment the invention relates to a water-soluble haemostatic preparation comprising as active ingredients a polyelectrolyte matrix, said matrix comprising at least one polymeric carboxylic acid of a predetermined average molecular weight range, and a bactericidal agent.

The said polymeric carboxylic acid is particularly polymeric α,β-unsaturated carboxylic acid, and preferably poly(acrylic acid), poly(methacrylic acid) and poly(crotonic acid) and mixtures thereof. The concentration of the polymeric matrix in the preparation of the invention is from about 0.5% to about 5% by weight, particularly from 0.5% to 2% by weight and preferably 1% by weight. When employing poly(acrylic acid), its average molecular weight is at least about 1,000, particularly from about 1,000 to about 100,000, and preferably from about 1,000 to about 10,000.

Poly(acrylic acid) having an average molecular weight of about 2,000 is most preferred.

The bactericidal agent in the preparation of the invention is selected from free and coordinated silver ions. Thus, the preparation may contain dissolved silver ions, oligomeric silver clusters, silver colloidal nanoparticles, preferably of a size of from about 0.5 nm to about 5 nm, and any mixture thereof. The preparation of the invention preferably contains from about $1x10^{-5}$mole per liter to about 1 mole per liter silver.

The preparation of the invention has a pH between about 3 and about 10, particularly from about 6 to about 10, and preferably a pH substantially equal to human blood physiological pH.

In another embodiment the invention relates to a method for the production of a water-soluble haemostatic bactericidal preparation comprising a poly(acrylic acid) of a predetermined average molecular weight higher than 1,000, preferably of from about 1,000 to about 100,000 and silver as a bactericidal agent, comprising the steps of (a) dissolving in water a poly(acrylic acid) having a molecular weight higher than 1,000, preferably from about 1,000 to about 100,000; (b) adjusting the pH of the solution obtained in (a) to a predetermined pH by adding a suitable physiologically acceptable base; (c) introducing a silver salt into solution obtained in (b); and optionally drying the solution obtained in step (c).

The method of the invention may further comprise the step of continuously or non-continuously irradiating the solution obtained in step (c) for a period of time determined by a predetermined change in the colour of the solution. Irradiation may be with visible, UV or combined visible-UV light, for example with full light of a mercury lamp. The colour change may be from colourless to blue, whereby a preparation containing oligomeric silver

clusters is obtained, or from colourless or blue to yellow, whereby a preparation containing silver nanoparticles is obtained.

In yet another aspect, the invention relates to a method of treating haemorrhage in a patient in need of such treatment comprising applying to the haemorrhage source a composition of the invention or a composition produced by the method of the invention.

Brief Description of the Figure

Figure 1: Effect of Arpocryl treatment on hematocrit in a 50% tail resection rat model. T represents time in minutes; Hcrt represents hematocrit; diamonds represent sham; dark squares represent untreated; light squares represent treatment with Arpocryl solution containing 1% PAA. *- P<0.05 vs. '- 0 in all groups.

Figure 2: Effect of Arpocryl treatment on bloodloss in a 50% tail resection rat model. TBL represents Total Blood Loss; Sh represents sham; UT represents untreated; Arp1% represents Arpocryl solution with 1% PAA. *- P<0.05 between Arp1% and UT.

Figure 3: Effect of Arpocryl treatment on blood pressure in a 50% tail resection rat model. T represents time in minutes; MAP represents mean arterial pressure; diamonds represent sham; dark squares represent untreated; light squares represent treatment with Arpocryl solution containing 1% PAA. *- between Arp1% and UT.

Figure 4: Effect of Arpocryl treatment on heart rate in a 50% tail resection rat model. T represents time in minutes; HR represents heart rate; diamonds represent sham; dark squares represent untreated; light squares represent treatment with Arpocryl solution containing 1% PAA. *- between Arp1% and UT.

Figure 5: Effect of Arpocryl treatment on blood lactate in a 50% tail resection rat model. T represents time in minutes; BL represents blood lactate; diamonds represent sham; dark squares represent untreated; light

squares represent treatment with Arpocryl solution containing 1% PAA. *- between Arp1% and UT.

Figure 6: Effect of Arpocryl treatment on base excess in a 50% tail resection rat model. T represents time in minutes; BE represents base excess; diamonds represent sham; dark squares represent untreated; light squares represent treatment with Arpocryl solution containing 1% PAA. *- between Arp1% and UT.

Figure 7: Effect of Arpocryl treatment on bicarbonate level in a 50% tail resection rat model. T represents time in minutes; BBi represents blood bicarbonate; diamonds represent sham; dark squares represent untreated; light squares represent treatment with Arpocryl solution containing 1% PAA. *- between Arp1% and UT.

Figure 8: Effect of Arpocryl treatment on $PACO_2$ in a 50% tail resection rat model. T represents time in minutes; diamonds represent sham; dark squares represent untreated; light squares represent treatment with Arpocryl solution containing 1% PAA. *- between Arp1% and UT.

Detailed Description of the Invention

The present invention relates to a preparation having combined antibacterial and haemostatic properties, which has storage stability and long shelf-life.

The haemostatic agent comprised in the preparation of the invention is a polyelectrolyte matrix which comprises at least one polymeric carboxylic acid of a predetermined average molecular weight range. The polymeric carboxylic acid is preferably a polymeric α,β-unsaturated carboxylic acid, particularly such as poly(acrylic acid), poly(methacrylic acid) or poly(crotonic acid), with poly(acrylic acid) being particularly preferred. The preparation may comprise a single such acid, or a mixture of several α,β-unsaturated carboxylic acids.

The concentration of the acid is preferably from about 0.5% to about 5% by weight, particularly from 0.5% to 2% by weight, with a concentration of 1% by weight being particularly preferred.

In preparations containing poly(acrylic acid) as the polymeric matrix, the average molecular weight of the poly(acrylic acid) is at least about 1,000, particularly from about 1,000 to about 100,000, and preferably from about 1,000 to about 10,000. Poly(acrylic acid) having an average molecular weight of about 2,000 is particularly preferred.

The bactericidal agent is an ionic silver releasing species. By the term "ionic silver releasing species" is meant any species that is capable of releasing silver ions into an aqueous solution containing the preparation of the invention. Particular ionic silver releasing species are dissolved, free silver ions, Ag^+, which may be generated by soluble silver salts, such as silver nitrate or silver acetate, present in the water-soluble preparation of the invention; oligomeric silver clusters; silver colloidal nanoparticles; and any mixture thereof. The term "oligomeric silver cluster" as used herein is to be taken to mean substantially oligomeric silver cluster ions, also referred to as coordinate ions, such as Ag_2^{1+}, Ag_4^{2+}, Ag_8^{2+} and the like. The term "silver nanocrystals" as used herein is to be taken to mean substantially metallic silver nanoparticles, preferably of a size of from about 0.5 nm to about 5 nm. The production of preparations containing these species will be described in more detail hereafter. Preparations containing monomeric silver ions Ag^+ will also be referred to hereafter as "Arpocryl" preparations. Preparations containing oligomeric cluster silver will also be referred to hereafter as "Clustacryl" preparations. Preparations containing colloidal silver nanoparticles will also be referred to hereafter as "Nanocryl" preparations

Preferred embodiments contain from about $1x10^{-5}$ mole per liter to about 1 mole per liter silver.

The pH of the haemostatic preparation of the invention is preferably a pH between about 3 and about 10, for example between about 6 and about 10, and particularly a pH that substantially equal to human blood physiological pH, i.e. about 7.3-7.6.

The haemostatic preparations of the invention may be in liquid form, particularly aqueous solutions having long shelf life. The haemostatic preparations of the invention may also be in dry, preferably freeze-dried form, for use by dissolution in pure water before use, to give a liquid preparation. The term "pure water" as used herein means distilled, deionized or otherwise purified water, which may be sterilised, and is physiologically compatible. The freeze-dried preparations may be stored over prolonged periods of time.

The liquid as well as dried preparations may be incorporated in bandages or the like, or applied directly to a wound or to body tissue. The possibility of directly applying the preparations of the invention to wounds and body tissues, for example during operation, is one of their major advantages.

As described above, the preparations of the invention preferably comprise poly(acrylic acid) as the haemostatic agent, and silver ion releasing material as the bactericidal agent. Thus, the silver may be in a variety of forms including ions, oligomeric clusters and nanocrystals. The poly(acrylic acid) binds with the silver, has a stabilising effect on the silver and prevents its precipitation. In some embodiments, the poly(acrylic acid) will trap the silver at the blood clot, slowly releasing silver ions, to provide long term, controlled antibacterial effect. This stabilising effect confers long shelf life to the haemostatic, antibacterial preparations of the invention, which is another major advantage.

The present invention thus provides a haemostatic material having a strong haemostatic and bactericidal action that produces no known side effects in the course of its application. Sustained release of the silver ions is not only achieved by the polymer trapping the silver at the blood clot. Rather, the Clustacryl and Nanocryl forms may be used as sustained release bactericidal haemostatic preparations, the Ag^+ ions being slowly released from the clusters and nanocrystals.

In a further aspect, the invention relates to a method for the production of a water-soluble haemostatic bactericidal preparation comprising a polyelectrolyte matrix, the matrix comprising a polymeric carboxylic acid, and a bactericidal agent. The method comprises dissolving the acid in water, adjusting the pH of the obtained solution, and addition the bactericidal agent. The obtained solution may optionally be dried, particularly freeze-dried.

In a particular embodiment, the method of the invention relates to the production of a water-soluble haemostatic bactericidal preparation comprising poly(acrylic acid) of a predetermined average molecular weight higher than 1,000, preferably of from about 1,000 to about 100,000, as the haemostatic agent, and silver as the bactericidal agent, the method comprising the steps of (a) dissolving in water a poly(acrylic acid) having a molecular weight higher than 1,000, preferably from about 1,000 to about 100,000 and particularly 2,000; (b) adjusting the pH of the solution obtained in (a) to a predetermined pH by adding a suitable physiologically acceptable base; (c) introducing a silver salt into solution obtained in (b); and optionally drying, particularly freeze-drying, the solution obtained in step (c).

The method of the invention may further comprise the step of continuously or non-continuously irradiating the solution obtained in step (c) for a period of time determined by a predetermined change in the colour of the solution.

Irradiation may be performed with visible, UV or combined visible-UV light, for example by full light of a mercury lamp.

By irradiating the solution up to a colour change from colourless to blue, a preparation containing oligomeric silver clusters is obtained. By irradiating the solution up to a colour change from colourless to yellow, a preparation containing silver nanoparticles is obtained. Preparations containing silver nanoparticles may also be prepared from preparations containing oligomeric silver clusters, by irradiating the cluster solutions up to a colour change from blue to yellow. Also when preparing irradiated solutions, the resulting preparations may be optionally freeze-dried for long storage.

The methods of the invention are described in detail in the following Examples.

In yet a further aspect, the invention relates to a method of treating haemorrhage in a patient in need of such treatment comprising applying to the haemorrhage source a composition of the invention.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

These and other aspects of the invention will be described in more detail on hand of the following Examples, which are descriptive only and do not in any sense limit the invention, which is only defined by the appended claims.

Examples

Example 1

ARPOCRYL preparations (Ag^+ preparations)

Preparation No. 1

The powder of poly(acrylic acid) of the average molecular weight of approximately 2,000 is dissolved in water so that the concentration is 1.10% by weight. The solution is then sterilised in advance by heating it to 100-130°C at increased pressure (approximately 2 atmospheres) and cooled to ambient temperature. Then 60 µL of 1M per liter $AgNO_3$ solution were added to 10 mL of the aforementioned poly(acrylic acid) solution. The pH of the solution was adjusted to 7.4 using concentrated KOH solution in water. This solution may be freeze-dried for storage and then re-dissolved in distilled water for use.

Preparation No. 2

Thirty µL of 1M silver acetate solution are added to 10 mL of sterile 5mM solution of poly(acrylic acid), having an average molecular weight of approximately 10,000. The solution is neutralized by adding aqueous KOH. The resulting solution may be freeze-dried for storage and then dissolved in distilled water for use.

Preparation No. 3

Poly(acrylic acid) (70 grams) having an average molecular weight of approximately 2,000 was dissolved in 1 liter of water and left to stand for 24 hours. The solution was then sterilised by heating to 100-130°C under elevated pressure (ca. 2 atmospheres). After cooling the sterilised solution to ambient temperature, the pH was adjusted to 7.5 by the addition of solid sodium hydroxide. 150 g of silver nitrate were dissolved in distilled water and added dropwise to the continuously stirred adjusted solution of poly(acrylic acid). The resulting solution may be freeze-dried for storage and then dissolved in pure water for use.

Example 2

Clustacryl Preparations (containing oligomeric silver clusters)

Preparation No. 4

The powder of poly(acrylic acid) of the average molecular weight approximately 2,000, is dissolved in water so that the concentration is 1.10% by weight and left to stand for 24 hours. The solution is then sterilised by heating it to 100-130°C at increased pressure (approximately 2 atmospheres), and cooled to ambient temperature. Then 60 μL of 1M per liter $AgNO_3$ solution were added to 10 mL of the aforementioned poly(acrylic acid) solution. The pH of the solution was adjusted to 7.4 using concentrated KOH solution in water. The solution was irradiated for 3 minutes by full light of the high pressure 250 W mercury lamp in silica cell with optical path length of 1 cm. During the irradiation, the reaction mixture became intensively blue coloured due to the photo-reduction of silver cations and the formation of polymer-stabilised charged oligomeric clusters, such as Ag_4^{2+}, having characteristic spectral absorption bands at approximately 290nm and 750nm. This solution may be freeze-dried for storage and then redissolved in distilled water for use.

Preparation No. 5

The procedure of Preparation 3 is repeated using poly(acrylic acid) of a molecular weight of 10,000. 0.1 mL of 1M silver acetate solution is added to 10 mL of sterile 10^{-2}M solution of poly(acrylic acid), having an average molecular weight of approximately 10,000. The pH of the solution is adjusted to 8 by the addition of concentrated aqueous KOH. The solution may be irradiated for 3 minutes by unshielded exposure to the full light of a high pressure 250 W mercury lamp in silica cell, having an optical path-length of 1 cm, to give an intensive blue solution containing polymer-stabilised charged oligomeric silver clusters. This solution may be freeze-dried for storage and then dissolved in distilled water for use.

Example 3

Nanocryl Preparations (containing with silver nanoparticles)

Preparation No. 6

The powder of poly(acrylic acid) of the average molecular weight approximately 2,000 is dissolved in water so that the concentration is 1.10% by weight and left to stand for 24 hours. The solution is then sterilised in advance by heating it to 100-130°C at increased pressure (approximately 2 atmospheres), and cooled to ambient temperature. Then 60μL of 1M per liter $AgNO_3$ solution were added to 10 mL of the aforementioned poly(acrylic acid) solution. The pH of the solution was adjusted to 7.4 using concentrated KOH solution. The solution was then irradiated for 10-20 minutes by full light of the high pressure 250 W mercury lamp in silica cell with optical path-length of 1 cm. During the irradiation, the reaction mixture became yellow coloured due to the photo-reduction of silver cations and formation of silver nanoparticles having characteristic spectral absorption bands at approximately 380 and 460 nm. This solution may be freeze-dried for storage and then redissolved in distilled water for use.

Preparation No. 7

0.1 ml of 1 M silver acetate solution are added to 10 ml of sterile 10^{-2}M solution of poly(acrylic acid) having average molecular weight of approximately 10,000. Aqueous KOH and acetic acid solutions were added to the solution to adjust the pH to 8. The solution was subsequently irradiated for 10-20 minutes by exposure to the unshielded radiation from a high pressure 250 W mercury lamp in silica cell, having an optical path length of 1 cm. During the irradiation, the reaction mixture became yellow coloured due to the photo-reduction of silver cations and subsequent formation of silver nanoparticles having characteristic spectral absorption bands at approximately 380 and 460 nm. This solution may be freeze-dried for storage and then dissolved in distilled water for use.

Preparation No. 8

The solution of Clustacryl (Preparation No. 4), was irradiated for 10-20 minutes by exposure to an unshielded high pressure 250 W mercury lamp in silica cell having an optical path-length of 1 cm. During the irradiation the reaction mixture became yellow coloured due to the photo-reduction of oligomeric silver clusters and silver ions and subsequent formation of silver nanoparticles having characteristic spectral absorption bands at approximately 380 and 460nm. This solution may be freeze-dried for storage and subsequently dissolved in pure water for use.

Preparation No. 9

The solution of Clustacryl, (Preparation No. 5) is irradiated for 10-20 minutes by the unshielded light of the high pressure 250 W mercury lamp in silica cell having an optical path length of 1 cm. During the irradiation, the reaction mixture became yellow coloured due to the photo-reduction of oligomeric silver clusters and silver ions and formation of silver nanoparticles having characteristic spectral absorption bands at approximately 380 and 460 nm. This solution may be freeze-dried for storage and then dissolved in pure water for use.

Example 4

Haemostatic Effect of Arpocryl in Rat Tail Model

Material and methods

Seventy adult male rats Sprague-Dawley weighing 290-320 g were enrolled in the project. Animal care and all experiments were performed in accordance to the National Research Council's *Guide for the Care and Use of Laboratory Animals*. The animals were anesthetized by intramuscular injection of 1.5 mg/kg dehydrobenzperidol and 24 mg/kg ketamine, and anesthesia was maintained by additional doses as necessary. Polyethylene catheters (PE-50) were introduced into the carotid artery for blood pressure and pulse measurements and blood sampling. The arterial line containing a calibrated pressure transducer was directly connected to a Controlled Data

Acquisition System (Cyber Amp 380, Axon Instruments, Foster City CA). Pulse rate was computed from the arterial tracing. Blood hematocrit was measured by a hematocrit centrifuge. Blood gases and acid-base state was determined by amperometric method (Compact 2 AVL Analyzer). Blood level of lactic acid was measured by spectophotometry (Sigma Diagnostics).

Hemorrhagic shock was induced by cutting of 50% of the rats tail length. Immediately after, the cut animals were randomly divided into 7 groups: untreated hemorrhage (UT, n=12); hemorrhage treated with topic application of Arpocryl containing 1% poly(acrylic acid) (Arp-1%, n=12); 3% of poly(acrylic acid) (Arp-3%, n=13); or 5% of poly(acrylic acid) (Arp-5%, n=12). Bled tail cuts were introduced into preweighed glass beakers and immersed in 2 ml of the assayed solution (treated animals) or normal saline (untreated animals) for all time of experiments. All the treatment solutions used contained 1.1% by weight of $AgNO_3$. After 4 hours or immediately after the death of an animal and shed blood was weighed. The amount of blood loss was determined by subtracting the beaker weight. Total blood loss was calculated as percent of blood volume, which was considered as 6.0 mL/100g body weight.

The mean arterial pressure (MAP), heart rate and hematocrit were determined just before (time = 0) and at 15, 20, 30, 60, 120, 180, and 240 min after tail resection. PaO_2, $PaCO_2$, pH, base excess, blood bicarbonates and blood lactate were determined at time points: 0, 60 and 240 min of the experiment. Data are presented as mean ± SEM. Kruskal-Wallis analysis of ranks was used to determine if a variable changed significantly with respect to time. Differences between groups (dependent and independent variables) were evaluated using Student' *t* and Wilcoxon rank-sum (Mann-Whitney U) tests. The cumulative survival was analyzed by the Kaplan-Meier assay (Log rank test). Mortality rates were compared using Yates corrected Chi-square test. A value of $p<0.05$ was considered statistically significant.

Results

Tail resection in untreated (UT) group resulted in a fall of mean arterial pressure (MAP) from 108.0 ± 2.3 to 42.9 ± 4.8 mmHg ($p< 0.01$), and a drop in heart rate from 399 ± 10 to 260 ± 21 bpm ($p< 0.01$) in 15 minutes. A similar drop in MAP was observed in all treated groups, except Arp-1% group. During the same period of time the hematocrit in UT group decreased from 41.9 ± 0.9 to 33.3 ± 1.4% ($p< 0.001$). After initial dropping, MAP spontaneously stabilized, and in 60 min was 51.2 ± 5.3 mmHg, and the hematocrit was 35.6 ± 1.5%. The plasma level of lactic acid (blood lactate) increased from 1.2 ± 0.2 to 2.0 ± 0.3 mM. The base deficit (BE) and PaO_2 values remained on the normal level during the first hour of bleeding. $PaCO_2$ value decreased after 60 min of bleeding from 40.7 ± 0.4 to 33.5±1.4 mmHg. This was consistent with reduced level of blood bicacbonate (HCO_3). During next three hours MAP, heart rate, hematocrit, $PaCO_2$ and PaO_2 values were stabile. The base deficit increased during next three hours to – 5.7 ± 1.2 mM ($p< 0.05$), and the level of blood lactate to 3.2 ± 0.1 mM ($p< 0.02$). HCO_3 decreased to 18.0 ± 0.7. Total blood loss in 4 hours was 30.4 ± 2.6% of blood volume, mean survival time was 229.6 ± 7.7 min, and mortality rate was 16.7%.

Topic application of Arpocryl solution with different concentrations of poly(acrylic acid) resulted in similar changes in MAP, heart rate, hematocrit, blood lactate, gases and acid-base state after 60 min of follow up, although animals treated by Arp-1% have demonstrated milder hypotension and only minimal changes in $PaCO_2$, HCO_3 and hematocrit. Total blood loss in 4 hours was 10.8 ± 1.6% of blood volume (Arp-1%, $p<0.0001$ *vs.* UT), 21.4 ± 2.6% (Arp-3%, $p<0.002$ *vs.* Arp-1%), 23.7 ± 2.5% (Arp-5%, $p<0.001$ *vs.* Arp-1%), mean survival time was 240 min in all treated groups (mortality rate was 0). Results are presented in Tables 1 and 2 and Figures 1 to 8.

Conclusions

Topic application of Arpocryl containing 1% of poly(acrylic acid), following resection of 50% of tail and haemorrhagic shock resulted in a significant decrease in bleeding, and improved haemodynamic and metabolic parameters as compared to a placebo group. Arpocryl containing 1% of poly(acrylic acid) (PAA) induces haemostasis at the site of application. The Arpocryl preparation with 1% PAA was more potent than Arpocryl preparations containing 3% and 5% PAA.

Table 1
Blood Loss After 50% Tail Resection and Treatment with Arpocryl Solutions

#	Untreated	Arpocryl 1% PAA	Arpocryl 3% PAA	Arpocryl 5% PAA
1	45.8	22.0	15.4	27.3
2	39.7	15.8	19.1	19.3
3	31.1	3.9	9	17.7
4	30.4	16.3	5	22.3
5	32.5	3.5	29.4	39.1
6	29.5	14.1	26.7	20.6
7	22.8	10.8	14.0	23.4
8	14.9	5.8	19.9	4.9
9	43.1	10.9	35	25.4
10	28.7	9.1	21.4	33.2
11	24.7	10.8	20.5	30.2
12	21.8	6.8	26.3	20.6
13			36	
MEAN	30.42	10.82	21.36	23.67
STD	9.04	5.53	9.29	8.61
STERR	2.61	1.60	2.58	2.49
TTEST	vs. UT	1.9E-06	0.022	0.074
TTEST	vs. 1%		0.002	2.6E-04
TTEST	vs. 3%			0.527

Survival time

All treated animals = 240 min

Untreated	
240	240
175	240
240	240
240	240
240	240
240	240
240	240
240	240
180	240
240	240
240	240
240	240
229.58	240.00
24.3514	0
7.7006	0
TTEST	0.16646
MANWHIT	0.448

Example 5

Inhibition of Bacterial Growth

An Arpocryl solution containing 1% poly(acrylic acid) and 1% $AgNO_3$ was tested for its capability of inhibiting growth of bacteria, in accordance with the NCCLS guidelines for agar dilution method M10-S9 January 1999 (NCCLS, Wayne, PA, USA). This solution was identical with the 1% poly(acrylic acid) Arpocryl preparation used in Example 4. In short, bacterial strains were thawed, innoculated on blood agar plate and then sub-cultured in Mhuller Hinton broth for 4-6 hours. The turbidity of the broth was then adjusted to 5x108cfu/ml and inoculated on Mhuller Hinton plates containing the antibiotic drugs or Arpocryl solution in the appropriate concentrations.

126 bacterial strains from the clinical culture collection of the Department of Clinical Microbiology if the Chaim Sheba Medical Center, Israel were tested. All bacterial strains were multi-resistant and represent the resistant flora of a tertiary medical center. The bacteria included different strains of *Staphylococcus aureus* MRSA, *Staphylococcus aureus* MSSA, *Enterococcus Spc.* VRE, *Enterococcus Spc.*, Escherichia coli, *Klebsiella pneumonia*, *Enterobacter Spc.* and *Pseudomonas aeruginosa*. Strains were frozen at -70°C in 15% glycerin until used.

MIC for clinically relevant antibiotic drugs (kept as powder until used) was performed for each of the tested strains (results not shown).

The Arpocryl solution was kept in a light protected container. It was then serially doubly diluted (1, 2, 4, 8, 16, 32, 64 etc., up to a dilution of 1024), to yield a final concentration in agar of 0.5 to 0.000001%.

Bacterial strains were inoculated on agar plates with the above mentioned concentrations of Arpocryl or antibiotic drugs and incubated overnight at 37°C. Results were read and documented.

All bacterial strains were inhibited by Arpocryl solution containing 1% AgNO3, and by its dilutions of up to 1:1024. The tested Arpocryl preparation is thus exhibited potent bacterial inhibitory activity against all of the tested strains and its inhibitory effect was preserved even at very high dilutions of its original 1% silver nitrate concentration.

Claims:

1. A water-soluble haemostatic preparation comprising as active ingredients a polyelectrolyte matrix, said matrix comprising at least one polymeric carboxylic acid of a predetermined average molecular weight range, and a bactericidal agent.

2. The preparation as claimed in claim 1, wherein said polymeric carboxylic acid is a polymeric α,β-unsaturated carboxylic acid.

3. The preparation as claimed in claim 1 or claim 2, wherein said polymeric α,β-unsaturated carboxylic acid is selected from the group consisting of poly(acrylic acid), poly(methacrylic acid) and poly(crotonic acid).

4. The preparation as claimed in any one of claims 1 to 3, wherein the concentration of said polymeric carboxylic acid is from about 0.5% to about 5% by weight.

5. The preparation according as claimed in claim 4, wherein the concentration of said polymeric carboxylic acid is from 0.5% to 2% by weight, preferably 1% by weight.

6. The preparation as claimed in any one of claims 2 to 5, wherein the average molecular weight of the poly(acrylic acid) is at least about 1,000.

7. The preparation as claimed in 6, wherein the molecular weight of the poly(acrylic acid) matrix is from about 1,000 to about 100,000, preferably from about 1,000 to about 10,000.

8. The preparation as claimed in claim 6, wherein the molecular weight of the poly(acrylic acid) is about 2,000.

9. The preparation as claimed in claim 1, wherein said bactericidal agent is selected from free and coordinated silver ions.

10. The preparation as claimed in claim 9, wherein said bactericidal agent is selected from the group consisting of dissolved silver ions, oligomeric silver clusters, silver colloidal nanoparticles and any mixture thereof.

11. The preparation as claimed in claim 10, containing from about $1x10^{-5}$mole per liter to about 1 mole per liter silver.

12. The preparation as claimed in claim 10 or claim 11, wherein the said silver nanoparticles are of the size of from about 0.5 nm to about 5 nm.

13. The preparation as claimed in any one of claims 1 to 12, having a pH between about 3 and about 10.

14. The preparation as claimed in claim 13, having a pH substantially equal to human blood physiological pH.

15. A method for the production of a water-soluble haemostatic bactericidal preparation comprising a poly(acrylic acid) of a predetermined average molecular weight higher than 1,000, preferably of from about 1,000 to about 100,000 and silver as a bactericidal agent, comprising:

(a) dissolving in water a poly(acrylic acid) having a molecular weight higher than 1,000, preferably from about 1,000 to about 100,000;

(b) adjusting the pH of the solution obtained in (a) to a predetermined pH by adding a suitable physiologically acceptable base;

(c) introducing a silver salt into solution obtained in (b); and

(d) optionally drying the solution obtained in step (c).

16. A method as claimed in claim 15, further comprising the step of continuously or non-continuously irradiating the solution obtained in step (c) for a period of time determined by a predetermined change in the colour of the solution.

17. The method as claimed in claim 16, wherein the irradiation is with visible, UV or combined visible-UV light.

18. The method as claimed in claim 17, wherein the solution is irradiated by full light of a mercury lamp.

19. A method as claimed in any one of claim 16 to 18, wherein said colour change is from colourless to blue, whereby a preparation containing oligomeric silver clusters is obtained.

20. A method as claimed in any one of claims 16 to 18, wherein said colour change is from colourless to yellow, whereby a preparation containing silver nanoparticles is obtained.

21. A method as claimed in claim 19, wherein the solution is further irradiated to give a colour change from blue to yellow, whereby a preparation containing silver nanoparticles is obtained.

22. A method as claimed in any one of claims 16 to 21 further comprising the step of drying the aqueous solutions obtained thereby.

23. A method as claimed in any one of claims 15 to 22, wherein said drying is by freeze-drying.

24. A method of treating haemorrhage in a patient in need of such treatment comprising applying to the haemorrhage source a composition as claimed in any one of claims 1 to 14 or a composition produced by the method of any one of claims 15 to 22.

INTERNATIONAL SEARCH REPORT

Inte ional Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/78 A61K33/38 A61P7/04 A61P31/04 //(A61K31/78, 33:38)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data, MEDLINE, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 241 179 A (ROHTO PHARMA) 14 October 1987 (1987-10-14) column 7, line 9 - line 12 claims 1-3 ---	1-8,13, 14
X	WO 98 06260 A (CAPELLI CHRISTOPHER C) 19 February 1998 (1998-02-19) abstract page 25, line 25 - line 26 claims 1-68 ---	1-3,9,15
X	WO 99 08691 A (SPACCIAPOLI PETER ;NELSON ERIC (US); PERIODINTIX INC (US); FRIDEN) 25 February 1999 (1999-02-25) abstract page 13, line 22 - line 25 claim 23 ---	1-15
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RU 2 056 843 C (LOPYREV VALENTIN ALEKSANDROVIC) 27 March 1996 (1996-03-27) & DATABASE WPI 51 Derwent Publications Ltd., London, GB; AN 1996-516763 abstract	1-24
A	US 4 215 106 A (ANNENKOVA VALENTINA M ET AL) 29 July 1980 (1980-07-29) abstract claims 1,2	1-24

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